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OF THE

GERMICIDAL CONSTITUENT OF BLOOD-SERUM.

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THE NATURE OF THE GERMICIDAL CONSTITUENT OF BLOOD-SERUM.[1]

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As early as 1872, Lewis and D. Cunningham[2] demonstrated the fact that bacteria injected into the circulation rapidly disappear. In the blood of twelve animals that had been treated with such injections, bacteria could be found after six hours in only seven. Of thirty animals, bacteria were found after twenty-four hours in the blood of only fourteen, and of seventeen animals, bacteria were found in only two when the examination was made from two to seven days after the injection.

In 1874, Traube and Gschiedlen[3] found that arterial blood, taken under antiseptic precautions from a rabbit into the jugular vein of which one and one-half c.c. of a fluid rich in putrefactive germs

[1] Read by title before the Medical Section of the First Pan-American Medical Congress, Washington, September 5, 1893.

[2] Eighth Annual Report of the Sanitary Commission of the Government of India.

[3] Schlesische Gesellschaft f. Vaterländ. Cultur, 1874.

had been injected forty-eight hours previously, failed to undergo decomposition for months. These investigators attributed the germicidal properties of the blood to its ozonized oxygen. Similar results were obtained by Fodor[1] and by Wysokowicz.[2] The latter accounted for the disappearance of the germs, not by supposing that they were destroyed by the blood, but that they found lodgment in the capillaries.

The first experiments made with extra-vascular blood were conducted by Grohmann[3] under the direction of A. Schmidt in his researches upon the cause of coagulation. It was found that anthrax-bacilli, after being kept in plasma, were less virulent, as was demonstrated by their effect upon rabbits. Grohmann supposed that in some way the bacteria were influenced by the process of coagulation.

In 1877, Fodor[4] made a second contribution to this subject, and in this he combated the retention-theory of Wysokowicz. One minute after the injection of one c.c. of anthrax-culture into the jugular vein, in eight samples of blood, Fodor found only one colony of the bacillus. Then he took the blood from the heart with a sterilized pipet and added anthrax-bacilli to it. This was kept at 38° C., and plates made from time to time showed a rapid diminution in the number of germs; after a time, when the blood had lost its germicidal properties, the number of germs began to increase.

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[1] Archiv f. Hygiene, B. 4.

[2] Zeitschrift f. Hygiene, B. 1.

[3] Ueber die Einwirkung des zellenfreien Blutplasma auf einige pflanzliche Mikroörganismen, Dorpat, 1884.

[4] Deutsche medicinische Wochenschrift, 1887.

In 1888, Nuttall,[1] working under the direction of Flügge, used defibrinated blood taken from various species of animals, rabbits, mice, pigeons, and sheep, and found that this blood destroyed the bacillus anthracis, bacillus subtilis, bacillus megaterium, and staphylococcus pyogenes aureus, when brought in contact with them. He also confirmed the further finding of Fodor that after a while the blood loses its germicidal properties and becomes a suitable culture-medium in which the germs grow abundantly.

Nissen[2] continued this work under Flügge's direction and reached the following conclusions:

(1) The addition of small quantities of sterilized salt-solution or bouillon to the blood does not destroy its germicidal properties.

(2) Cholera-germs and Eberth's bacilli are easily destroyed by fresh blood.

(3) For a given volume of blood there is a maximum amount of bacilli which can be destroyed.

(4) Blood whose coagulability has been destroyed by the injection of peptone is still germicidal.

(5) Blood in which coagulation is prevented by the addition of 25 per cent. of magnesium sulfate has its germicidal properties decreased.

(6) Filtered blood-plasma from the horse is germicidal.

Behring[3] has attributed the action of the blood of the white rat on anthrax-bacilli to its great alkalinity. He has made a number of titrations by which he shows that the blood-serum of the white

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[1] Zeitschrift f. Hygiene, B. 4. [2] Ibid., B. 6. [3] Ibid., B. 6.

rat is somewhat more alkaline than that of certain animals that are more susceptible to anthrax, such as the rabbit, the guinea-pig, and the cow. His deduction is not justified, because there are many other and more important points in which these animals differ more markedly from the white rat than in slight differences in the alkalinity of the blood-serum. Had he shown that the blood of the adult rat, which is not susceptible to anthrax, is more alkaline than that of the young rat, which is susceptible, his argument would have been more plausible; but even then it would not have deserved the dignity of positive evidence.

In 1890, Buchner,[1] aided by Voit, Sittmann, and Orthenberger, made a most valuable contribution to our knowledge of the germicidal properties of blood. The results of this work are stated as follows:

1. The germicidal action of blood is not due to phagocytes, because it is not influenced by the alternate freezing and thawing of the blood, by which the leukocytes of the rabbit are destroyed.

2. The germicidal properties of the cell-free serum must be due to its soluble constituents.

3. Neither neutralization of the serum, nor the addition of pepsin, nor the removal of carbon-dioxid gas, nor treatment with oxygen has any effect upon the germicidal properties of the blood.

4. Dialysis of the serum against water destroys its activity, while dialysis against 0.75 per cent. salt-solution does not. In the diffusate there is no germicidal substance. The loss by dialysis with

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[1] Archiv f. Hygiene, B. 10.

water must be due to the withdrawal of the inorganic salts of the serum.

5. The same is shown to be the case when the serum is diluted with water and when it is diluted with the salt-solution. In the former instance the germicidal action is destroyed, while in the latter it is not.

6. The inorganic salts have in and of themselves no germicidal action. They are active only in so far as they affect the normal properties of the albuminates of the serum. The germicidal properties of the serum reside in its albuminous constituents.

7. The difference in the effects of the active serum and that which has been heated to 55° C. is due to the altered condition of the albuminate. The difference may possibly be a chemical one (due to changes within the molecule) or it may be due to alterations in mycelial structure. The albuminous bodies act upon the bacteria only when the former are in active state.

We wish at this point to call attention to an inconsistency between the results obtained by Buchner and the conclusions that he draws. In experiment No. 45 he renders the serum slightly acid and adds 0.1 gram of pepsin to each five c. c. of serum (showing by a side experiment that this pepsin actively digests coagulated egg-albumin in neutral solution) and finds that the digestive action of the pepsin does not lessen the germicidal properties of the serum. In fact he states this in his conclusions, but his ultimate opinion, and the one held by him in his latest contribution, is that the germicidal constituent of the blood is the serum-albumin. How much serum-

albumin remains in blood-serum after it has been thoroughly digested with pepsin? He could scarcely have chosen a more positive method of demonstrating that the germicidal constituent is not serum-albumin. Either his pepsin was not active, and on this supposition his experiment is without value, or the active constituent of blood-serum is a substance that is not destroyed or materially altered by peptic digestion. We know that the peptones not only have no germicidal properties, but that they belong to that class of proteids that is most favorable to the growth and development of germs. We recognize this fact when we add peptones to the various artificial media on which we cultivate germs. However we shall return to this subject. At present we shall proceed with the literature of the subject.

The successful researches of Buchner led many other investigators to enter this field of experimentation, and some of them have made valuable contributions to our knowledge of the germicidal action of the blood under varying conditions, but so far as the nature of the germicidal constituent is concerned but little or no progress has been made. Prudden[1] found that ascitic and hydrocele fluids restrain the development of certain germs. Rovighi[2] reported that the germicidal action of the blood is increased in febrile conditions. Pekelharing[3] enclosed anthrax-spores in bits of parchment and introduced them under the skin of rabbits. Thus treated, the spores soon lost their virulence and

[1] Medical Record, 1890.
[2] Atti della Accad. Med. di Roma, 1890.
[3] Ziegler's Beiträge, B. 8.

finally their capability of growth. The destruction of these spores could not have been due to phagocytes, which did not penetrate the parchment, but must have been caused by soluble poisons. Behring and Nissen[1] found that the serum of the white rat. the dog, and the rabbit destroys anthrax-bacilli. while serum obtained from the mouse, sheep, guinea-pig, chicken, pigeon, and frog, has no such action. It will be observed from this that there is no constant relation between the germicidal action of the blood of animals of different species and their susceptibility to the disease caused by the germ. Thus, the rabbit is highly susceptible to anthrax, notwithstanding the fact that its blood destroys large numbers of these germs. On the other hand, the chicken is immune to anthrax from the moment when it comes from the shell, and yet the bacillus anthracis grows luxuriantly in the extra-vascular blood of the chick. This demonstrates that there is a great difference between the action of extra-vascular blood and that circulating in the body, constantly fed, and in case of need, altered in composition by certain glands.

Halliburton has prepared from the lymphatic glands a globulin which he designates as cell-globulin β, and which agrees with fibrin-ferment in inducing coagulation in plasma. Hankin[2] has tested the germicidal properties of this cell-globulin. His experiments have been conducted in the following manner: The lymphatic glands (in later experiments

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[1] Zeitschrift f. Hygiene, B. 8.

[2] Centralblatt f. Bakteriologie, B. 9.

the spleen also) of a dog, or of a cat, are freed as much as possible from fat and connective tissue, then finally divided and extracted with dilute solution of sodium sulfate (one part of a saturated solution to nine parts of water). The cell-globulin passes into solution, while the other proteids are but sparingly soluble. After twenty-four hours, the fluid is filtered and mixed with an excess of alcohol. The voluminous precipitate containing the cell-globulin is collected on a filter and washed with absolute alcohol. For use, a part is dissolved in water, and a small quantity of a bouillon-culture of the anthrax bacillus is added. From time to time plate-cultures are made, along with control-plates, and in this way the germicidal properties of the substance are demonstrated. Hankin closes this contribution with the following conclusions:

1. Halliburton's cell-globulin β has marked germicidal properties.

2. In this respect it differs from fibrin-ferment.

3. The germicidal properties of this substance seem to be identical with those of serum as described by Buchner, Nissen, and Nuttall.

4. The active properties of the serum are probably due to this or to an allied body.

Bitter[1] has repeated the experiments of Hankin, but fails to confirm them. Bitter states that he has followed Hankin's directions exactly. However this may be, it is certain that the spleen contains a germicidal substance, but whether it can be extracted by the method of Hankin or not we do not know.

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[1] Zeitschrift f. Hygiene, B. 12.

That the germicidal constituent of the spleen is identical with Halliburton's cell-globulin β or with any other globulin, we very much doubt. It certainly is a nuclein, and it is altogether possible that Hankin obtained traces of this nuclein in his extracts. In this case the extract would show, or fail to show, germicidal properties according to the relative amounts of nuclein and other substances present. The less globulin and the more nuclein present the more marked would the germicidal effect be.

Christmas[1] has prepared a germicidal substance from the spleen and other organs by the following method:

The animal is killed with ether, opened under antiseptic precautions and the organ removed, cut into fine pieces, covered with fifty cubic centimeters of glycerin and allowed to stand for twenty-four hours, and then filtered. The filtrate is precipitated with five times its volume of alcohol, and this fluid is immediately decanted. The precipitate is washed with absolute alcohol in order to remove the glycerin. Then the traces of alcohol are removed by pressure and the precipitate dissolved in twenty-five cubic centimeters of distilled water. Through this solution air is driven for some hours in order to destroy the traces of alcohol. Then the fluid is filtered and its germicidal action tested.

Bitter has also examined this method, and the impartial reader must see that he has not done so with fairness. However, this fact renders the work all the more valuable because his results confirm the

[1] Annales de l'Institut Pasteur, t. v.

statements of Christmas. Bitter killed his animals by venesection, and, in some cases at least, prepared the substance in unsterilized vessels; but even when this was done the solution was germ-free and manifested marked germicidal properties. Bitter finally finds a difference between this substance and the germicidal constituent of blood-serum; the latter, he states, is certainly destroyed by a temperature of 65°, while the solution of Christmas, after having been heated to this temperature, is still capable of destroying from 35,000 to 40,000 typhoid-bacilli within four hours. Buchner,[1] in his latest contribution to the subject has the following to say in condemnation of Christmas:

"A method given by Christmas for the preparation of germicidal solutions from the organs of normal rabbits has also been tested by Bitter. Germicidal solutions were indeed obtained, which, however, differed materially from active serum, for in three experiments, notwithstanding heating to 65° C., the germicidal action remained."

It is altogether possible that the more powerful action of the solution made by Christmas is due to the fact that it contained the germicidal substances in more nearly a chemically pure condition than they exist in blood-serum. It is also highly probable that the arrest of the germicidal activity of blood-serum by a temperature of 55° C. is not due to the destruction of its germicidal constituent, but is due to the action of the heat on other constituents of the fluid.

[1] Archiv f. Hygiene, B. 17.

Some attempts have been made to determine the nature of the germicidal constituent by the action of precipitating reagents on the proteids of blood-serum. In his latest contribution, Buchner states that he has not been able to obtain a germicidal solution by precipitating all the proteids with absolute alcohol, freeing the precipitate from alcohol, drying it, and then redissolving. He does not give the methods employed in freeing the precipitate from alcohol, the temperature or the conditions under which it was dried, or the nature of the menstruum by which resolution was effected. In the absence of these needed details, his conclusion that alcohol destroys the germicidal substance must remain open to question. On the other hand, Christmas states that when the proteids are precipitated with alcohol and the precipitate dissolved in a volume of water equal to that of the original serum, the solution thus obtained has a more powerful germicidal action than the serum. Bitter in an experimental review of the statement of Christmas gives the following detailed account of one experiment:

"Ten cubic centimeters of serum were poured into fifty cubic centimeters of alcohol (strength of alcohol not given), stirred, and the precipitate immediately separated from the alcohol by filtration. (He fails to state whether or not sterilized filter-paper was used.) The precipitate was freed from alcohol by pressure between folds of filter-paper (again he fails to state whether or not this paper was sterilized), then dried at 37° C., and mixed with ten cubic centimeters of sterilized distilled water. On being allowed to stand for a short time at 37° C., nearly all

of the precipitate was redissolved. The solution was then separated from the deposit by filtration (through unsterilized filter paper?) and tested."

It can scarcely be a matter of surprise that Bitter found germs nearly always present in the solution obtained in this careless manner. However, he did find that the germs present did not develop when the solution was kept at 37° C., and, moreover, that germs added to this solution were destroyed. Bitter concludes that in truth anthrax and typhoid bacilli are destroyed by "precipitated serum," but not so energetically as by normal serum.

Emmerich, Tsuboi, Steinmetz, and Löw[1] have made interesting and valuable contributions relating to the effect of precipitation of the proteids upon the germicidal action of blood-serum. An active serum was dialyzed in a sterilized parchment-paper tube against water for from twelve to eighteen hours. By the expiration of that time the serum-globulin, becoming insoluble on account of the withdrawal of inorganic salts, was deposited. The dialyzer was dried with sterilized filter paper and the globulin-free serum was precipitated with several volumes of alcohol. The precipitate was collected on a sterilized "falten-filter" and the alcohol removed from the precipitate by sterilized porous plates and filter-paper. The precipitate was then finely divided, dried for half an hour in vacuo at 36° C., then rubbed up in a sterilized mortar and dissolved in sterilized water, to which salt-solution had been added. In the solution thus prepared germs did not show, after

[1] Centralblatt f. Bakteriologie, B. 12.

from three to four hours, either a marked crease in or decrease, but when the solution was heated to 100° C., allowed to cool, and then inoculated with germs, the increase was four-hundred-fold within four hours. It was next found that, if instead of water, a 0.05 per cent. aqueous solution of potassic hydrate was employed in dissolving the alcoholic precipitate in the globulin-free serum, this solution possessed all the germicidal strength of the original serum. The same was found to be true of dilute alkaline solutions of the alcohol precipitate in serum from which the globulin had not been removed. The dilute alkali was shown not to have any germicidal action in and of itself. From these experiments the investigators mentioned conclude that the germicidal constituent of blood-serum is an alkaline compound of serum-albumin. They also found that heating the serum-albumin alkaline solution to 65° C., or higher, destroyed its germicidal action, and they explain this effect of heat on blood-serum and on their artificial solution by supposing that the high temperature breaks up the combination of the alkali with the serum-albumin. Furthermore, they found that a serum that had been rendered inactive by a temperature of 55° C. could be regenerated in part at least by the addition of the small amount of alkali mentioned.

Since Fodor[1] and Zuntz[2] have shown that freshly-drawn blood rapidly decreases in alkalinity on standing *in vitro*, an explanation of the fact that

[1] Centralblatt f. Bakteriologie, B. vii.
[2] Centralblatt f. med. Wissenschaft, 1867.

blood-serum rapidly loses its germicidal properties naturally suggests itself. Emmerich and his co-workers confirm their belief in this theory by demonstrating that blood-serum that has been rendered very feebly acid (0.67 part of sulphuric acid per mille) has no germicidal action, but furnishes a good culture-medium.

The foregoing investigations are very valuable, inasmuch as they show the important rôle that the small amount of alkali plays in the germicidal action of blood-serum. This had, indeed, already been demonstrated by Fodor[1] by a quite different line of investigation. This experimenter found that the resistance of rabbits to anthrax is markedly increased by the administration, by the stomach or subcutaneously, of sodium phosfate, carbonate, or bicarbonate, or of potassium carbonate.

Löw concludes that the introduction of the alkali into the albumin-molecule increases its liability, and he cites examples from organic chemistry in support of this view.

There are some additional points of interest in the theory of Emmerich and his assistants. As has been stated, they believe that the serum-albumin is the germicide, but they think it highly probable that only a comparatively small part of the albumin is active, and this small part, they suppose, originates in the albumin of the daily food, which is converted into lymph-cells, and by the disintegration of these it passes into solution in the blood. They admit, however, that there are some reasons for believing,

[1] Centralblatt f. Bakteriologie, B. vii.

with Buchner, that the whole of the serum-albumin is active. They state that it is possible, *but highly improbable*, that the germicidal substance is not the serum-albumin, but some substance that is precipitated along with this by alcohol and other agents.

We hope to show that the germicidal agent is not serum-albumin and that this "highly improbable" substance does exist.

In a short and somewhat unsatisfactory review of the report of Emmerich and his co-workers, Buchner[1] devotes himself to a consideration of the question of the regeneration of serum rendered inactive by heating to 55° C. on the addition of an alkali. He details one experiment made by himself on this point. The experiment confirms the work of Emmerich, but Buchner offers an interpretation that is wholly theoretic and by no means convincing. He finds that the regenerated serum, when heated to 60° C., still has a retarding effect upon the growth of germs, and he argues from this that the germicidal action of the "regenerated serum" is (for some unknown reason) due to its being less suited to the growth of bacteria. No one knows better than Buchner the influence of various chemical substances on the temperature at which an active serum is converted into an inactive form, and yet he overlooks altogether the possible effect of increased alkalinity on this conversion. Had he heated the regenerated serum to 100° C. he would then have found that it forms a very fertile culture-medium.

Hankin[2] has recently published a paper that is

[1] Centralblatt f. Bakteriologie, B. xii. [2] Ibid.

more valuable in its suggestions than in its experimental details. He suggests that the germicidal substance is a special secretion of the eosinophile granular cells. The granular matter in these cells is, according to his theory, the antecedent of the germicidal substance.

There are many other minor contributions to this subject, but those mentioned contain all the essential points, and there is no necessity for a further review of the literature. It is true that Aronson[1] has very recently announced to the Berlin Medical Society that he has isolated a powerful antitoxin from the blood-serum of animals rendered immune to diphtheria, and that with this substance he has cured guinea-pigs infected with this disease. Following the example of another illustrious German investigator, he refuses to tell how this curative substance is prepared. It is needless to say that this manner of dealing with scientific investigations has not as yet found favor with the unsophisticated profession in the new world.

From a careful and critical study of the investigations that have been briefly reviewed, we have come to the following conclusions:

1. The serum-albumin is not the germicidal substance in blood-serum. As has been stated, either this must be true or the experiment by which Buchner demonstrated that an active pepsin does not destroy the germicidal action of blood-serum must have been an error; because peptic digestion readily and completely converts serum-albumin into

[1] Berliner klin. Wochenschrift, 1893.

peptones, and we know that peptones are especially favorable to bacterial growth.

2. The germicidal substance must belong to the proteids. Otherwise it would be difficult to explain the fact that a temperature of 55° C. renders blood-serum inactive.

3. The only proteid likely to be present in blood-serum and which is not destroyed by peptic digestion is nuclein.

Having reached these conclusions, the following questions naturally present themselves:

1. Is there a nuclein in blood-serum?

2. Has this nuclein, if there be one, germicidal properties?

These questions we have attempted to answer.

Dogs and rabbits were the animals from which the serum was obtained. Healthy animals that had not previously undergone any experimentation were selected. The animal was firmly fixed in a holder, the carotid was laid bare under antiseptic precautions. A ligature and a small clamp were applied to the artery about two inches apart, the former distad, and the latter centrad. Then a slit in the artery was made with a sterilized knife, and a small sterilized glass canula, with sterilized and dried rubber tube leading into a sterilized Erlenmayer flask, was introduced into the artery and held in place by another ligature. Then the clamp was removed and the blood flowed into the flask. In each case the animal was bled to death. The flask containing the blood was placed in the ice-chest and allowed to remain for twenty-four hours. By

the expiration of this time, a wine-colored serum had separated. This serum was poured into a second sterilized flask and about ten volumes of a mixture of equal parts of absolute alcohol and ether were added. This produced a voluminous precipitate that was nearly white. This was allowed to stand twenty-four hours, and in some cases much longer, the alcohol and ether twice, or more often, during the time, being decanted and replaced by equal volumes. Then the supernatant fluid was decanted and an equal volume of a 0.2 per cent. solution of hydrochloric acid containing active pepsin was added, and the flask placed in an incubator at 38° C. and the digestion was continued until the fluid failed to respond to the biuret test for peptones. Each time this test was made the fluid was decanted from the undigested portion and replaced by an equal volume of fresh digestive fluid. In some instances the flask containing this fluid was allowed to stand in the incubator for several days. This was not necessary in order to complete the digestion, but was allowed as a matter of convenience. In all cases the digestion was prompt and proceeded to a certain point, when it ceased altogether. The undigested portion was small in amount and grayish in color. This was collected on a small sterilized filter and washed first with 0.2 per cent. solution of hydrochloric acid, and then with alcohol. After the washing with alcohol, the filter was allowed to stand exposed to the air for half an hour or longer in order that all of the alcohol might pass through or evaporate. The precipitate was then dissolved in a sterilized solution of potassic hydrate. The strength of

this alkaline solution usually employed was 0.12 per cent. Usually this solution contained in addition to the alkali 0.6 per cent. of sodium chlorid. In some instances a solution containing 1.2 grams of potassic hydrate, 6 grams of sodium chlorid, and 1 gram each of sodium bicarbonate and disodium hydrogen phosfate to one liter of water was employed as a solvent. The solution was filtered through a Chamberland tube and received in a sterilized flask.

The solution thus obtained was perfectly clear, colorless, and did not respond to the biuret test. The addition of strong nitric acid produced a cloudiness, which dissolved on the further addition of the acid. This acid solution did not become yellow on being heated, but did so after the addition of ammonia.

We have now answered the first question. Blood-serum contains a nuclein. We hope to investigate at some time in the future the relation between this nuclein and fibrin-ferment.

The origin of the nuclein found now for the first time in blood-serum is an interesting question. Does it come from the disintegration of the polynuclear cells, or shall we regard certain white blood-corpuscles as unicellular organs whose function it is to secrete this nuclein?

In proceeding to determine whether or not this nuclein has germicidal properties, the solution was distributed in sterilized test-tubes, five c.c. being placed in each tube. It should be stated that in dissolving the nuclein, the volume of the solvent employed was in all cases the same as that of the

blood-serum from which the nuclein was obtained. These tubes were inoculated with different germs and plates made at varying intervals of time, in order to test the germicidal action. One and the same platinum loop was used in the preparation of each plate.

EXPERIMENT I.

A nuclein-tube was inoculated with the bacillus of *Asiatic cholera*, and plates made from this gave the following results:

Time,	Immediately	5 min.	15 min.	30 min.	1 hr.	1½ hr.	22 hrs.
No. of colonies,	2100	43	54	71	90	115	1200

That the alkali in which this nuclein was dissolved did not cause the decrease in the number of germs is shown by the subsequent increase.

EXPERIMENT II.

Staphylococcus pyogenes aureus.

Time,	Immediately	1 hr.	4 hrs.	7 hrs.	24 hrs.
No. of colonies,	4000	1720	1050	810	0

EXPERIMENT III.

Anthrax bacillus without spores.

Time,	Immediately	1 hr.	4 hrs.	7 hrs.	24 hrs.
No. of colonies,	100	43	10	1	0

EXPERIMENT IV.

Cholera-germ.

Time,	Immediately	1 hr.	4 hrs.	7 hrs.	24 hrs.
No. of colonies,	470	45	1	0	410

It may be stated that the final increase in the number of cholera-germs occurred both in the nuclein-solution prepared from the serum of the rabbit and that prepared from the serum of the dog.

EXPERIMENT V.

Staphylococcus pyogenes aureus.

Time,	Immediately	1 hr.	5 hrs.	19 hrs.	24 hrs.
No. of colonies,	Countless	22,000	12,525	155	0

EXPERIMENT VI.

Anthrax-bacillus without spores.

Time,	Immediately	1 hr.	5 hrs.	19 hrs.	24 hrs.
No. of colonies,	1120	165	0	0	0

All of the foregoing experiments were made with the solution of nuclein in sterilized water containing 0.12 per cent. potassic hydrate and 0 6 per cent. of sodium chlorid. The following were made in the other solution mentioned. It may be stated that the culture of the aureus experimented with retained its vitality for days in water containing 0.5 per cent. of potassic hydrate.

EXPERIMENT VII.

Staphylococcus pyogenes aureus.

Time,	Immediately	1 hr.	4 hrs.	7 hrs.	24 hrs.
No. of colonies,	5000	2500	1600	1200	0

EXPERIMENT VIII.

Anthrax-bacillus without spores.

Time,	Immediately	1 hr.	4 hrs.	7 hrs.	24 hrs.
No. of colonies,	43	7	0	0	0

EXPERIMENT IX.

Cholera-bacillus.

Time,	Immediately	1 hr.	4 hrs.	7 hrs.	24 hrs.
No. of colonies,	350	105	150	42	0

EXPERIMENT X.

Staphylococcus pyogenes aureus.

Time,	Immediately	1 hr.	5 hrs.	19 hrs.	24 hrs.
No. of colonies,	Countless	25,000	5525	65	500

EXPERIMENT XI.

Anthrax-bacillus without spores.

Time,	Immediately	1 hr.	5 hrs.	19 hrs.	24 hrs.
No. of colonies,	430	0	0	0	0

We have made many other tests of the germicidal action of the nuclein obtained from blood-serum, but as all of them gave practically the same results, further repetition is unnecessary.

We have also made many experiments on the effect of heat and other agents on the germicidal action of this nuclein, but we prefer to report these later, as we have obtained some unexpected results. Suffice it to say that while boiling destroys the germicidal action, the temperature to which these solutions may be heated and still show some retarding action on germs has surprised us.

The fact that the germicidal constituent of blood-serum can be isolated has an important practical bearing. Blood-serum therapy has proved impracticable on account of the large amount of the fluid which must be injected. Nuclein-therapy now promises to enable us to avoid this difficulty, and possibly the near future may find us using this agent in the treatment of disease. The nuclein may be obtained from an animal rendered immune to diphtheria, and a sufficient quantity of this injected into the blood or under the skin of a child suffering with this disease may effect a cure, but we will not prophecy. The future will tell us what it has in store when the future shall have become the present.